The volatile leaf oil of *Juniperus microsperma* and its taxonomy

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ABSTRACT

The composition of the volatile leaf oil of *Juniperus microsperma* is reported and compared with the oils of *J. convallium, J. davurica* var. *arenaria, J. sabina, J. saltuaria* and *J. semiglobosa*. The volatile leaf oil of *J. microsperma* from Song Zong, Xizang is very distinct and is dominated by sabinene (33.9%), pregeijerene B (16.3%), elemol (14.6%) and 8-α-acetoxyelemol (7.1%) with moderate amounts of terpinen-4-ol, germacrene D, and α- and β-eudesmols. There appears to have been such long genetic separation between *J. microsperma* and *J. davurica* var. *arenaria*, *J. semiglobosa* and *J. sabina* that the terpenoids have evolved different patterns due to selection. Thus, the phylogenetic relationships are not discernable in the volatile oil compositions. Published on-line: **www.phytologia.org** *Phytologia 95(1): 87-93(Feb. 1, 2013).*

KEY WORDS: *Juniperus microsperma, J. sabina, J. davurica* var. *arenaria, J. saltuaria, J. semiglobosa, J. convallium, J. convallium var. microsperma,* terpenoids.

The taxonomy of *Juniperus microsperma* (W. C. Cheng & L. K. Fu) R. P. Adams has been unstable. It was originally described as *Sabina convallium* (Rehder & E. H. Wilson) W. C. Cheng & L. K. Fu var. *microsperma* W. C. Cheng & L. K. Fu (Acta Phytotax. Sin. 13(4): 86, 1975) then raised to *Sabina convallium* W. C. Cheng & L. K. Fu W. C. Cheng & L. K. Fu (Fl. Xizangica 1: 390, 1983). The genus *Sabina* was not recognized so it was changed to *Juniperus convallium* var. *microsperma* (W. C. Cheng & L. K. Fu) Silba (Phytologia Mem. 7: 33, 1984). Then more recently raised to the specific level as *Juniperus microsperma* (W. C. Cheng & L. K. Fu) R. P. Adams (Biochem. Syst. Ecol. 28: 540, 2000).

Farjon (2005, 2010) recognized *J. convallium* var. *microsperma*, but Adams (2011) recognized *J. microsperma*. Recently, Mao et al (2010) collected materials of *J. microsperma* near the type locality in Song Zong, Xizang (Tibet) and included this collection in a phylogeny of *Juniperus* based on cpDNA sequences. They found that their sample of *J. microsperma* was not related to *J. convallium* (a turbinate cone juniper), but was part of a clade with *J. sabina* and *J. semiglobosa* and junipers from North America (Fig. 1). The multi-seeded *sabina* junipers have seed cones that are oval, ellipsoid, round, reniform or irregular globose with one to several seeds per cone.

The inclusion of *J. microsperma* in *J. convallium* has been problematical because, although some of its seed cones have pointed tips, so that they appear to be turbinate; other cones are oval or globose (Fig. 2). Comparing the seed cones of *J. microsperma*, *J. sabina* var. *arenaria* (now *J. davurica* var. *arenaria*, see Adams and Schwarzbach, 2012), and *J. convallium* (Fig. 3), one can see the similarity in some seed cones. However, the DNA data seems clear that *J. microsperma* is not a member of the turbinate junipers that include *J. convallium*, but rather part of the sabinoid junipers (Fig. 1).

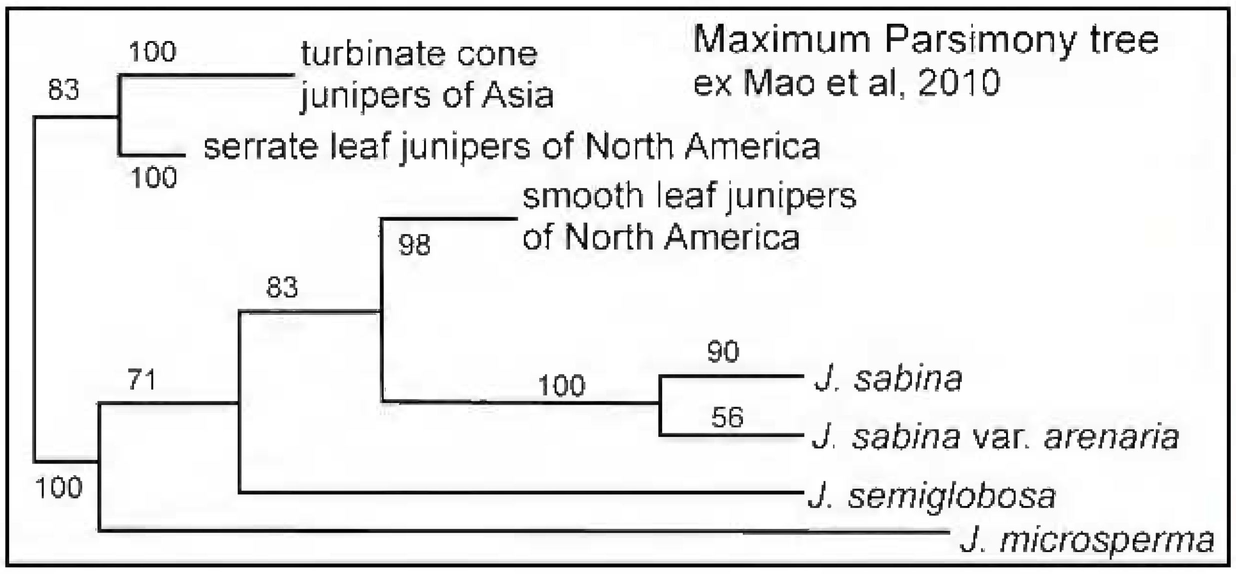

Figure 1. A maximum parsimony tree showing the clade with *J. microsperma, J. sabina* and smooth leaf junipers of North America. the numbers at the branch points are bootstrap support values (adapted from Mao et al., 2010).

Fig. 2. Seed cones of *J. microsperma* from *J-Q Liu QTP-2011-201* specimen.

Figure 3. left: *J. microsperma*, J-Q Liu 201; center: *J. sabina* var. *arenaria*; right: *J. convallium.*

The purpose of the present paper is to report on the leaf essential oil of *J. microsperma* from Xizang and compare it with its reputed nearest relatives *(J. semiglobosa*, *J. sabina* and *J. davurica* var. *arenaria*, Fig. 1) as well as *J. convallium* and *J. saltuaria* (members of the turbinate junipers). The leaf essential oil of these nearest relatives have been reported: *J. convallium* (Adams, Zhang and Chu, 1993a); *J. davurica* and var. *arenaria* (Adams et al., 1998; Adams, Nguyen and Liu, 2006); *J. saltuaria* (Adams, Zhang and Chu, 1993b), *J. semiglobosa* (Adams et al., 1992; Adams 1999).

MATERIAL AND METHODS

Specimens used in this study (species, location, collection numbers): *J. microsperma*, Song Zong, Xizang (Tibet), China, *Jian-Quan Liu QTP-2011-201(lab accession 13633*; *J. convallium*, Songpan, Sichuan, China, *Adams 8523-8525*; *J. davurica* var. *arenaria*, AR, sand dunes, Lake Qinghai, Qinghai, China, *Adams 10347-52*; *J. sabina*, TS, Tian Shan Mtns., Xinjiang, China, Adams 7836-38; *J. saltuaria*, 23 km se of Forestry Station, Gansu, China, *Adams 6788-6790*; Deqin Co., Yunnan, China, *Adams 8494-96, 8505; J. semiglobosa*, 60 km sw of Bishket, Kyrgystan, *Adams 8210-8212*, 2 km s of Dzhabagly, Kazakhstan, *Adams 8227, 8229, 8230.* Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU).

Fresh (100 g.) and air dried (10-15 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus with a diethyl ether floating trap (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The volatile leaf oil of *J. microsperma* from Song Zong, Xizang is very distinct and dominated by sabinene (33.9%), pregeijerene B (16.3%), elemol (14.6%) and 8-α-acetoxyelemol (7.1%) with moderate amounts of terpinen-4-ol, germacrene D, and α- and β-eudesmols (Table 1). Several compounds that are found in *J. davurica* var. *arenaria*, *J. semiglobosa* and *J. sabina,* such as linalool and 4-epi-abietal, are also found in *J. microsperma* (Table 1). However, several terpenes that seem to typify *J. davurica* var. *arenaria*, *J. semiglobosa* and *J. sabina,* such as cis-piperitol, citronellol, methyl geranate, and germacrene D-4-ol, are not found in *J. microsperma.* In fact, the terpenes of *J. microsperma* seem a little more similar to those of *J. saltuaria* in regards to pregeijerene B, elemol, α- and β-eudesmols, and 8-α-acetoxyelemol (Table 1).

The DNA sequence data is robust in showing *J. microsperma* in a clade with the sabinoid junipers (Fig. 1). Mao et al. (2010) (Table 2, Fig. 4) estimated the branch point where *J. microsperma* unites with *J. davurica* var. *arenaria*, *J. semiglobosa* and *J. sabina* at approximately 19 Mya (10-28 Mya). Although this may not be a long period to accumulate neutrally evolved intergenic and intron mutations, this is a very long time for adaptational changes in defense chemicals (terpenoids) to accumulate. As noted previously (Adams, 2011), the terpenoids provide their most useful taxonomic information at and below the species level. In the present case, there appears to have been such a long genetic separation between *J. microsperma* and *J. davurica* var. *arenaria*, *J. semiglobosa* and *J. sabina* that the terpenoids have evolved different patterns due to selection thus their phylogenetic relationships are not discernable in the volatile oil compositions.

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Table I. Comparisons of the per cent total oil for leaf essential oils for sabin = *J. sabina*, Tian Shan, China; aren = *J. davurica* var. *arenaria*, Qinghai, China; micro = *J. microsperma*, Xizang, China; Gansu; salt = *J. saltuaria*, Yunnan and conv = *J. convallium*. Components that tend to separate the species are highlighted in boldface.

KI	Compound	aren	semi	sabin	micro	salt	conv
921	tricyclene	-	-	-	-	t	0.1
924	α-thujene	0.9	1.5	0.9	0.9	0.9	0.1
932	**α-pinene**	**3.8**	**2.3**	**1.9**	**0.9**	**4.3**	**42.9**
945	α-fenchene	t	t	t	t	t	t
946	camphene	t	0.1	t	t	t	0.3
969	**sabinene**	**57.1**	**57.5**	**46.5**	**33.9**	**39.4**	**1.3**
974	β-pinene	t	0.2	t	0.2	0.1	0.8
988	**myrcene**	**3.4**	**3.1**	**3.6**	**0.5**	**1.2**	**8.1**
996	(methyl 4-methylhexanoate)	0.7	-	-	-	-	-
1001	δ-2-carene	0.2	-	0.1	0.5	0.2	-
1002	α-phellandrene	0.2	0.1	0.1	t	0.1	0.1
1008	δ-3-carene	0.2	t	0.1	-	t	t
1014	α-terpinene	0.9	1.3	0.9	1.0	1.3	0.1
1020	p-cymene	0.1	0.7	0.3	0.3	0.4	1.0
1024	**limonene**	**1.4**	**0.8**	**1.3**	**0.8**	**0.9**	**5.3**
1025	β-phellandrene	t	0.9	0.9	0.7	0.6	1.2
1036	1,8-cineole	-	-	-	-	-	t
1036	2-heptyl acetate	-	-	-	-	-	t
1044	(E)-β-ocimene	0.2	0.2	0.2	-	-	-
1054	γ-terpinene	1.5	2.0	1.4	1.8	2.2	0.2
1065	cis-sabinene hydrate	1.5	0.7	0.7	0.7	0.7	0.2
1074	trans-linalool oxide(furanoid)	-	-	-	-	-	t
1086	terpinolene	0.9	0.9	0.9	0.6	0.8	1.0
1087	2-nonanone	0.5	-	-	-	-	-
1095	trans-sabinene hydrate	0.9	0.6	0.3	0.5	0.8	0.1
1095	linalool	0.8	0.5	0.8	0.4	-	0.7
1100	nonanal	t	-	t	-	-	-
1101	cis-thujone (=α-thujone)	-	-	t	-	-	-
1102	isoamyl-isovalerate	0.2	-	-	-	-	-
1106	cis-rose oxide	-	t	-	-	-	-
1112	trans-thujone(=β-thujone)	-	0.1	0.3	-	-	-
1116	3-methyl-3-butenyl-isovalerate	0.5	-	-	0.1	0.1	-
1118	cis-p-menth-2-en-1-ol	0.3	0.3	0.2	0.3	0.3	0.2
1122	α-campholenal	-	-	-	-	-	0.1
1137	trans-sabinol	-	-	0.2	-	-	t
1137	trans-p-menth-2-en-1-ol	0.2	0.2	0.2	0.2	0.2	0.3
1137	trans-verbenol	-	-	-	-	-	0.3
1147	3-methyl-2-butenyl-3-methyl butanoate	-	-	-	0.1	-	-
1148	**citronellal**	**0.3**	**0.2**	**t**	-	-	**-**
1156	sabina ketone	-	0.1	-	-	-	-
1165	2-allyl-phenol, isomer	-	0.1	-	-	-	-
1165	borneol	-	-	-	0.3	-	-
1174	**terpinen-4-ol**	**3.2**	**4.4**	**3.0**	**2.0**	**4.9**	**0.3**
1179	p-cymen-8-ol	-	t	-	0.1	t	-
1186	α-terpineol	0.1	0.2	0.2	t	0.2	t
1193	(Z)-4-decenal	0.1	-	t	-	-	-

KI	Compound	aren	semi	sabin	micro	salt	conv
1195	**cis-piperitol**	**0.2**	**0.2**	**0.1**	**-**	**0.1**	**-**
1204	verbenone	-	-	-	-	-	0.2
1207	**trans-piperitol**	**t**	**0.1**	**0.1**	**0.1**	**0.1**	**-**
1219	coahuilensol, methyl ether	-	0.1	-	-	-	-
1223	**citronellol**	**1.7**	**0.7**	**0.1**	**-**	-	**-**
1232	**(3Z)-hexenyl-3-methyl butanoate**	**-**	**-**	**-**	**0.6**	t	**-**
1241	carvacrol, methyl ether	-	-	-	-	t	-
1249	piperitone	0.2	-	0.1	t	0.1	0.2
1255	(Z)-4-decen-1-ol	-	t	t	-	-	-
1257	linalyl acetate	0.3	-	0.4	-	-	-
1257	methyl citronellate	2.0	1.1	0.2	-	-	-
1274	**pregeijerene B**	**-**	**-**	**-**	**16.3**	**3.1**	**-**
1285	bornyl acetate	t	0.2	0.1	0.2	0.1	1.1
1287	trans-linalool oxide acetate	-	-	t	-	-	-
1290	**trans-sabinyl acetate**	**2.6**	**-**	**15.9**	**-**	-	**-**
1293	2-undecanone	1.2	0.2	-	-	-	0.3
1298	carvacrol	-	-	-	-	0.1	0.1
1315	(E,E)-2,4-decadienal	t	0.2	-	-	-	-
1320	**aromatic phenolic,<u>149</u>,91,134,164**	**-**	**-**	**-**	**-**	**0.3**	**1.0**
1322	**methyl geranate**	**0.7**	**0.4**	**0.5**	**-**	-	**-**
1345	α-cubebene	-	-	-	-	-	0.6
1346	α-terpinyl acetate	t	-	0.3	-	-	-
1350	citronellyl acetate	0.4	-	-	-	-	-
1374	α-copaene	-	-	-	-	-	0.1
1379	geranyl acetate	0.3	-	-	-	-	-
1387	β-bourbonene	-	-	-	0.1	-	-
1387	β-cubebene	-	-	-	-	-	1.0
1389	β-elemene	-	-	-	0.2	-	-
1410	α-cedrene	-	0.4	0.3	-	0.1	-
1417	**(E)-caryophyllene(β-caryophyllene)**	**-**	**-**	**-**	**0.8**	t	**0.2**
1419	β-cedrene	-	0.3	0.2	-	-	-
1429	cis-thujopsene	-	0.2	0.2	-	-	-
1448	cis-muurola-3,5-diene	-	-	-	-	-	1.3
1448	sesquiterpene, <u>43</u>, 105, 147, 220	-	-	-	0.8	-	-
1452	α-humulene	-	-	-	t	t	0.2
1469	n-dodecanol	-	-	-	-	0.6	
1475	trans-cadina-1(6),4-diene	-	-	-	-	-	1.1
1478	γ-muurolene	0.1	-	-	-	-	-
1480	**germacrene D**	**0.1**	**-**	**-**	**2.4**	-	**0.5**
1489	δ-selinene	-	-	-	t	-	-
1493	trans-murrola-4(14),5-diene	-	-	-	-	-	3.3
1493	epi-cubebol	0.1	-	-	-	-	1.2
1495	γ-amorphene	-	t	-	-	-	-
1500	α-muurolene	0.2	-	0.1	t	0.1	0.4
1513	γ-cadinene	0.6	t	0.2	t	0.2	-
1513	**cubebol**	**-**	**-**	**-**	**-**	-	**4.7**
1518	endo-1-bourbonol	0.1	-	-	-	-	-
1522	δ-cadinene	0.8	0.2	0.2	t	0.4	2.1
1528	zonarene	-	-	-	-	-	0.6
1533	trans-cadina-1,4-diene	-	-	t	-	-	0.5
1537	α-cadinene	0.1	-	t	-	-	-
1539	α-copaen-11-ol	-	-	-	0.3	-	-

KI	Compound	aren	semi	sabin	micro	salt	conv
1548	**elemol**	**t**	**0.1**	**0.1**	**14.6**	**3.8**	**-**
1559	germacrene B	t	0.1	-	0.2	0.1	-
1561	(E)-nerolidol	-	-	t	-	-	-
1565	(Z)-3-hexenyl benzoate	-	-	-	0.2	-	-
1574	**germacrene D-4-ol**	**3.5**	**0.3**	**0.6**	**-**	**0.5**	**0.4**
1587	trans-murrol-5-en-4-α-ol	-	-	-	-	-	0.6
1587	allo-cedrol	-	0.7	0.6	-	-	-
1600	**cedrol**	**-**	**14.3**	**13.2**	**-**	**1.6**	**0.1**
1607	β-oplopenone	0.3	t	0.1	-	-	t
1608	humulene epoxide II	-	-	-	-	-	0.1
1627	1-epi-cubenol	t	-	0.1	-	-	2.5
1630	γ-eudesmol	-	-	-	0.8	0.7	-
1632	α-acorenol	-	-	t	-	-	-
1638	epi-α-cadinol	0.4	t	0.1	0.2	0.2	t
1640	epi-α-muurolol	0.4	t	0.1	0.2	0.3	-
1644	α-muurolol	0.1	-	t	-	-	-
1645	cubenol	-	-	-	-	-	0.6
1649	**β-eudesmol**	**-**	**-**	**-**	**1.5**	**0.7**	**-**
1652	**α-eudesmol**	**-**	**-**	**-**	**1.4**	**0.8**	**-**
1652	α-cadinol	1.0	0.2	0.4	-	0.4	0.4
1670	bulnesol	-	-	t	0.5	0.4	-
1671	n-tetradecanol	-	-	-	-	0.3	-
1688	shyobunol	0.1	0.2	0.1	-	-	-
1740	(E,E)-2,6-farnesol	t	-	-	-	-	-
1792	**8-α-acetoxyelemol**	**-**	**-**	**-**	**7.1**	**2.4**	**-**
1901	epi-laurenene	-	-	-	-	-	-
1905	iso-pimara-9(11),15-diene	-	-	-	-	-	-
1907	**pimara-8(11),15-diene**	**-**	**-**	**-**	**-**	**0.7**	**-**
1958	**iso-pimara-8(14),15-diene**	**-**	**-**	**-**	**-**	**1.0**	**0.2**
1978	manoyl oxide	-	t	-	-	-	
1987	iso-pimara-7,15-diene	-	-	-	-	0.1	1.1
2055	**abietatriene**	**t**	**-**	**t**	**t**	**1.6**	**3.5**
2056	**manool**	**-**	**-**	**-**	**-**	**14.8**	**-**
2087	abietadiene	0.1	-	t	t	0.5	3.0
2132	**nezukol**	**-**	**-**	**-**	**-**	**1.3**	**-**
2282	sempervirol	-	-	-	-	0.3	0.2
2298	**4-epi-abietal**	**0.4**	**0.1**	**t**	**0.3**	-	**-**
2312	abieta-7,13-dien-3-one	1.2	-	0.1	-	-	-
2314	**trans-totarol**	**-**	**-**	**-**	**-**	**0.7**	**0.6**
2331	trans-ferruginol	t	-	-	-	t	t
2402	abietol	0.1	-	-	-	-	-

KI = Kovat's Retention Index on DB-5(=SE54) column using alkanes. Compounds in parenthesis ( ) are tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.